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Methods of Analysis by the U.S. Geological Survey National Water Quality Laboratory—Processing, Taxonomy, and Quality Control of Benthic Macroinvertebrate Samples

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METHODS OF ANALYSIS BY THE U.S. GEOLOGICAL SURVEY NATIONAL WATER QUALITY LABORATORY — PROCESSING, TAXONOMY, AND QUALITY CONTROL OF BENTHIC MACROINVERTEBRATE SAMPLES

By Stephen R. Moulton II, James L. Carter, Scott A. Grotheer, Thomas F. Cuffney, and Terry M. Short

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CONTENTS

TABLES

FIGURES

CONVERSION FACTORS, ABBREVIATIONS, AND DEFINITIONS

Multiply	By	To obtain
	Length	
micrometer (µm)	0.00003937	inch
millimeter (mm)	0.03937	inch
centimeter (cm)	0.3937	inch
meter (m)	3.281	foot
	Area	
square centimeter (cm^2)	0.1550	square inch
square meter (m^2)	10.76	square foot
	Volume	
liter (L)	0.2642	gallon
milliliter (mL)	0.0338	ounce, fluid
dram (dr)	0.0625	ounce, avoirdupois
	Mass	
gram (g)	0.03527	ounce, avoirdupois

Temperature Conversion

Degree Celsius (°C) may be converted to degree Fahrenheit (°F) by using the following equation:

$$°F = 9/5(°C) + 32$$

Abbreviations frequently used in this report

BG	Biological Group
BMI(s)	benthic macroinvertebrate(s)
CTA	Custom Taxonomic Assessment
NAWQA	National Water-Quality Assessment Program
NWQL	National Water Quality Laboratory
QA/QC	quality assurance/quality control
QC	quality control
RBP	Rapid Bioassessment Protocol
RTA	Rapid Taxonomic Assessment
SOP	standard operating procedure
STA	Standard Taxonomic Assessment
USEPA	U.S. Environmental Protection Agency
USGS	U.S. Geological Survey
T&E	threatened and endangered
>	greater than
<	less than
≤	less than or equal to
≥	greater than or equal to
±	plus or minus

Glossary

Density	The abundance of benthic macroinvertebrates per unit area.
High(er) taxonomic level	Levels of taxonomy, such as Class, Order, or Family; may also be used to indicate a relation (for example, Family is a *higher level* than Genus).
Large-rare	Large and generally rare organisms present in a sample that may or may not be accounted for in the sorted portion of a subsample.
Low(er) taxonomic level	Levels of taxonomy, such as Genus or Species; may also be used to indicate a relation (for example, Genus is a *lower level* than Family)
Unprocessed abundance	The actual number of organisms identified and enumerated for a taxon or sample; often referred to as “raw abundance.”
Remnant	The detrital portion of a sample that has been sorted.

Glossary—Continued

Sample abundance	The number of identified and enumerated organisms corrected for laboratory and field subsampling.
Sample preparation	Washing and sieving a sample prior to subsampling or sorting benthic macroinvertebrates.
Sorting	The removal of benthic macroinvertebrates from the sample matrix into coarse taxonomic groupings.
1-Stage Subsampling	A procedure to obtain randomly selected square-grid subsamples from the original sample.
Stage-1 subsampling frame	A gridded subsampling frame used to obtain square-grid subsamples from the original sample.
Stage-1 grid	A randomly selected square grid from a stage-1 subsampling frame.
Stage-1 subsample	The resulting composite of all sorted stage-1 grids.
2-Stage Subsampling	A procedure to obtain randomly selected square-grid subsamples from a stage-1 subsample.
Stage-2 subsampling frame	A gridded subsampling frame used to obtain square-grid subsamples from a stage-1 subsample.
Stage-2 grid	A randomly selected square grid from a stage-2 subsampling frame.
Stage-2 subsample	The resulting composite of all sorted stage-2 grids.
Taxon (pl. taxa)	A proper name given to a group of related organisms (for example, the Order Trichoptera, Family Hydropsychidae, Genus *Hydropsyche*, and Species *Hydropsyche simulans* Ross are taxa).
Visual sort	To sort organisms from a sample without magnification; as performed, the qualitative sample-processing method or the large-rare organism sort in the quantitative sample-processing method.

METHODS OF ANALYSIS BY THE U.S. GEOLOGICAL SURVEY NATIONAL WATER QUALITY LABORATORY — PROCESSING, TAXONOMY, AND QUALITY CONTROL OF BENTHIC MACROINVERTEBRATE SAMPLES

By Stephen R. Moulton II, James L. Carter, Scott A. Grotheer, Thomas F. Cuffney, and Terry M. Short

ABSTRACT

Qualitative and quantitative methods to process benthic macroinvertebrate (BMI) samples have been developed and tested by the U.S. Geological Survey's National Water Quality Laboratory Biological Group.

The qualitative processing method is based on visually sorting a sample for up to 2 hours. Sorting focuses on attaining organisms that are likely to result in taxonomic identifications to lower taxonomic levels (for example, Genus or Species). Immature and damaged organisms are also sorted when they are likely to result in unique determinations. The sorted sample remnant is scanned briefly by a second person to determine if obvious taxa were missed.

The quantitative processing method is based on a fixed-count approach that targets some minimum count, such as 100 or 300 organisms. Organisms are sorted from randomly selected 5.1- by 5.1-centimeter parts of a gridded subsampling frame. The sorted remnant from each sample is resorted by a second individual for at least 10 percent of the original sort time. A large-rare organism search is performed on the unsorted remnant to sort BMI taxa that were not likely represented in the sorted grids.

After either qualitatively or quantitatively sorting the sample, BMIs are identified by using one of three different types of taxonomic assessment. The Standard Taxonomic Assessment is comparable to the U.S. Environmental Protection Agency Rapid Bioassessment Protocol III and typically provides Genus- or Species-level taxonomic resolution. The Rapid Taxonomic Assessment is comparable to the U.S. Environmental Protection Agency Rapid Bioassessment Protocol II and provides Family-level and higher taxonomic resolution. The Custom Taxonomic Assessment provides Species-level resolution whenever possible for groups identified to higher taxonomic levels by using the Standard Taxonomic Assessment. The consistent use of standardized designations and notes facilitates the interpretation of BMI data within and among water-quality studies. Taxonomic identifications are quality assured by verifying all referenced taxa and randomly reviewing 10 percent of the taxonomic identifications performed weekly by Biological Group taxonomists. Taxonomic errors discovered during this review are corrected.

BMI data are reviewed for accuracy and completeness prior to release. BMI data are released phylogenetically in spreadsheet format and unprocessed abundances are corrected for laboratory and field subsampling when necessary.

INTRODUCTION

Purpose and Scope

Benthic macroinvertebrates (BMIs) are animals that live on or in the substrates (for example, sediments, woody debris, macrophytes, algae) of aquatic habitats, such as lakes and streams. Typical examples of BMIs are flatworms, snails and clams, segmented worms, crustaceans, and aquatic insects. BMIs are used more frequently in water-quality studies than any other group of organisms (Rosenberg and Resh, 1993, p. 4). BMI data are frequently used to develop biocriteria and rank aquatic habitats according to their biological health (for example, Hilsenhoff, 1982). When combined with

measurements of water chemistry and habitat, BMI data provide an integrated assessment of water quality in lakes and streams (Gilliom and others, 1995).

The U.S. Geological Survey's (USGS) National Water Quality Laboratory (NWQL) Biological Group (BG) processes BMI samples that have been collected by using a variety of techniques from diverse aquatic habitats throughout the United States. These samples vary greatly in the density of organisms and the types and amounts of detritus they contain. Therefore, the BG has developed methods for efficiently sorting and identifying BMIs from a complex array of sample matrices. Five main steps are used to process a BMI sample: (1) prepare a sample for subsampling or sorting; (2) sort BMIs from the sample matrix; (3) identify and enumerate BMIs; (4) enter data and calculate BMI abundances; and (5) apply quality-control (QC) procedures to quality assure (QA) steps (1) through (4).

Water-quality studies have a variety of data needs. Although often not explicitly stated, each study has its own data-quality objectives. The BG has developed well-defined qualitative and quantitative processing methods that are sufficiently flexible to satisfy most data-analytic methods currently (2000) used for including estimates of BMI community composition in water-quality studies.

The objective of the qualitative method is to produce a comprehensive and taxonomically accurate list of organisms contained in a BMI sample. Processing involves size-fractionating the sample into coarse and fine components. The entire coarse component is sorted. All or some part of the fine component is sorted, depending on the volume of the sample. Size-fractionation aids in sorting large, more fully developed BMIs that can be identified to lower taxonomic levels. Both components are visually sorted for up to, but not exceeding, a total of 2 hours.

The objective of the quantitative method is to estimate the abundance of each taxon sorted from a BMI sample. The method is similar to the fixed-count method described in Barbour and others (1999). Organisms are sorted by using X 10 magnification from either the entire sample or more often from randomly selected grid subsamples of the original sample. The quantitative method developed by the BG differs slightly from Barbour and others (1999) in four aspects:

(1) Instead of acquiring a fixed count of organisms with a numerical range of ±20 percent, the goal of the BG method is to acquire a minimum number of organisms. For example, if a fixed-count target was 300 organisms, by using the method of Barbour and others (1999), the number of organisms sorted could range from 240 to 360 (300 ±20 percent). In contrast, the method used by the USGS BG consists of sorting out at least 300 organisms. Athough these methods are similar, randomly sorting a minimum number of organisms provides a more uniform data set indexed to the fixed-count goal from which a rarefied (Hurlbert 1971), unbiased index of richness might be determined (Barbour and Gerritsen, 1996; Vinson and Hawkins, 1996; Larsen and Herlihy, 1998).

(2) When estimates of abundance are based on subsamples of the original sample, large-rare organisms are visually sorted from the unsorted portion of the sample for an additional 15 minutes. Sorting large-rare organisms from the unsorted portion of the sample provides a biased but more representative estimate of the taxa present in a sample (Vinson and Hawkins, 1996).

(3) The BG method limits sorting effort to a maximum of 8 hours. In general and in agreement with a previous finding by the U.S. Environmental Protection Agency's (USEPA) Rapid Bioassessment Protocol (RBP) (Plafkin and others, 1989), the BG has found that about 100 organisms can be sorted from BMI samples in 1 hour. However, samples that contain excessive amounts of detritus and that have organism densities near or less than a given fixed-count goal are extremely time-intensive to sort (for example, greater than 50 hours).

(4) The BG sorts all quantitative BMI samples under a dissecting microscope that uses X 10 magnification. Other laboratories that use a similar fixed-count method might sort without magnification.

Three levels of taxonomy are presented. The Standard Taxonomic Assessment (STA) is comparable to the USEPA RBP III (Barbour and others, 1999) and provides Genus-level and lower taxonomic resolution for most taxa. The Rapid Taxonomic Assessment (RTA) is comparable to the USEPA RBP II and provides Family-level and higher taxonomic resolution. Also described is a Custom Taxonomic Assessment (CTA) that provides nonstandard taxonomic resolution when a customer requests it.

The objectives of this report are as follows: (1) to provide detailed descriptions of the methods used by the BG at the NWQL to process qualitatively and quantitatively BMI samples; (2) to provide detailed procedures and information for the taxonomic identification of BMIs; and (3) to provide detailed procedures to quality assure the processing and identification of BMIs.

The sorting methods, taxonomic identification procedures, and quality-assurance and quality-control procedures described herein replace those presented by Cuffney and others (1993a) for BMI samples collected by the USGS National Water-Quality Assessment (NAWQA) Program.

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We thank Rob Plotnikoff (Washington State Department of Ecology), Evan Hornig (USGS Texas District), Barry Poulton (USGS Midwest Science Center), and Daniel Pickard (NWQL BG) for providing colleague reviews of the manuscript.

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ANALYTICAL METHOD

Benthic macroinvertebrate, processing procedures, B-9135-00 Parameter Codes

Qualitative visual sort, STA:
NWQL lab code 2176
300 organism count subsample, STA:
NWQL lab code 2172
100 organism count subsample, STA:
NWQL lab code 2174
100 organism count subsample, RTA:
NWQL lab code 2175

1. Chemicals, Equipment, and Supplies Necessary to Process Benthic Macroinvertebrate Samples

The following list of chemicals, equipment, and supplies are used to process BMI samples at the NWQL.

1.1. Chemicals

- 70-percent ethanol
- CMC-10™ mounting media
- Glycerin
- Potassium hydroxide

1.2. Equipment

- Compound microscopes (40 – 1000 X magnification)
- Dissecting microscopes (6 – 50 X magnification)
- Estimation trays (see Section 1.4.1)
- Fiber optic illuminators
- Hot plate
- Plastic wash basins
- Portable incandescent desk lamps
- Slide dryers
- Sonicator

- Standard metal sieves (mesh sizes = field collection mesh size)
- Subsampling frames (see Section 1.4.2)
- White sorting trays (various sizes, for example 15 by 20 cm, 20 by 30 cm, and 40 by 50 cm)

1.3. Supplies

- Forceps (jewelers, lightweight, blunt)
- Probes (fine tipped and blunt)
- Petri dishes
- Pasteur pipettes
- Plain microscope slides
- Cover slips
- Glass screw cap jars
- Screw cap polyseal vials (4–6 dram preferred)
- Shell vials (1/4 dram)
- Genitalia microvials
- Cotton
- Random numbers table
- Quantitative BMI Sample Processing—Subsampling and Preliminary Enumeration Worksheet (fig. 1) (Available in electronic spreadsheet format for ease of calculation and consistent recommendations for processing)
- Slide Preparations—Identification and Enumeration Worksheet (fig. 2)
- BMI Identification and Enumeration Bench Data Sheet (fig. 3)
- Slide labels (minimum information: sample identification code, name of taxonomist, year of identification, collection date, slide number)
- Sorting labels (minimum information: sample identification code, taxonomic sort category)
- Taxonomic identification labels (minimum information: taxonomic identification, name of taxonomist, year of identification, sample identification code, state, county, waterbody name, specific location, collection date, collector)
- Scissors
- Vial racks
- Scrub brushes

1.4. Construction of Subsampling Equipment

1.4.1. Estimation trays

Estimation trays are constructed of 3.2 mm thick clear Plexiglas™. Each tray is 1.3 cm deep and is etched on the bottom with grid lines at 1.3-cm intervals. Estimation tray dimensions are listed in table 1.

1.4.2. Subsampling frames

Subsampling frames are constructed of 1.3 cm thick clear Plexiglas™, 2.5- by 2.5-cm galvanized wire mesh, and 100-µm Nitex™ mesh. Although most samples are collected by using a mesh size >100 µm, a fine mesh facilitates removing grids of sample matrix because the tarsal claws of insects and other fine matter do not adhere to a fine mesh as easily as they do to a coarse mesh. The 100-µm mesh and the galvanized mesh are bonded to the bottom of the Plexiglas™ frame with silicone adhesive. The galvanized mesh supports the 100-µm mesh and functions as a reference grid for the removal of the 5.1- by 5.1-cm subsamples. Dimensions of the three subsampling frames are listed in table 1.

Quantitative BMI Sample Processing -- Subsampling and Preliminary Enumeration Worksheet

Sample ID: _____ Collection Date: _____ Reach: _____ Site ID: _____ Field Subsample: _____

Processed by : _____ E_s checked (count: _____) , subsampling frames, and paperwork checked by (initials): _____

Stage-1 subsampling frame		Stage-2 subsampling frame		Estimation tray		Correction factor
Stage-1 subsampling frame size:		Stage-2 subsampling frame size:		Estimation tray size:		*(W x Y)/(X x Z)*
Total grids in stage-1 subsampling frame (*W*)		Total grids in stage-2 subsampling frame (*Y*)		Total cells in estimation tray (*e*):		
Total stage-1 grids used (*X*)		Total stage-2 grids used (*Z*)		Total cells counted from tray:		

Stage-1 subsampling frame grid density estimation

Subsampling frame coordinates			Estimation tray coordinates/counts						Total count	*MGC*	*EGC*	Time
Grid No.	Row (R)	Column (C)	R/C	*C1*	R/C	*C2*	R/C	*C3*	*(C1+C2+C3)*	*(Total Count/ 3)*	*(MGC x e)*	
1												
2												
3												
4												
5												
Recommended processing scheme:										Total *(Σ)*		
										Average *(Σ/5)*		

Preliminary counts from individual sorted stage-1 or stage-2 grids

Grid no.	Row	Column	Count	Time	Grid no.	Row	Column	Count	Time	Grid no.	Row	Column	Count	Time
1					11					21				
2					12					22				
3					13					23				
4					14					24				
5					15					25				
6					16					26				
7					17					27				
8					18					28				
9					19					29				
10					20					30		Σ		

Figure 1. Example of worksheet used to record subsampling information for the quantitative processing of benthic macroinvertebrate samples. E_s = sorting effectiveness;
MGC = mean grid count for the 1.3 cm (centimeter) x 1.3 cm grids; EGC = estimated grid count for each select stage-1 grid.

Slide Preparations - Identification and Enumeration Worksheet

Sample Identification Code: Collection Date: Site ID: Reach:

State: County: Waterbody: Location: Elevation:

Prepared by: _____ Date: ___/___/_____ Time: _____hr(s)

Identified By: _____ Date: ___/___/_____ Time: _____hr(s)

Slide	Slip	MTD	LS	Subs	Ref	Position #1	Position #2	Position #3	Position #4
1	1								
	2								
2	1								
	2								
3	1								
	2								
4	1								
	2								
5	1								
	2								
6	1								
	2								
7	1								
	2								
8	1								
	2								
9	1								
	2								
10	1								
	2								
11	1								
	2								
12	1								
	2								
13	1								
	2								
14	1								
	2								
15	1								
	2								

All Slides and Taxa Accounted For _____ (initials)

Page ____ of ____

Figure 2. Example of worksheet used to record identifications of benthic macroinvertebrates prepared on microscope slides. ID, identification; hr(s), hour(s);MTD = number of organisms mounted; LS = life stage; Subs = subsample; Ref = reference slide.

BMI Identification and Enumeration Bench Data Sheet

Sample ID: Collection Date: Reach: Site ID: Field Split:

State: County: Waterbody: Location: Elevation:

Block Code: ____________________ **Method** (circle one): Qualitative 100 300 Other: __________________

Sort by : ______________________________________**Date**: ____/____/____**Prep Time** : ______hr(s)**Sort Time** : _______hr(s)

Non-Chironomid ID's by: ____**Date**: ____/____/____ **Time** : ___________ hr(s)

Chironomid Mount by : ____ **Date**: ____/____/____ **Time** : ___________ hr(s)

Chironomid ID's by : ____**Date**: ____/____/____ **Time** : ___________ hr(s)

			Correction factor(s)	
Taxon	**LS**	**Notes**	**1:1**	**:**

All Vials and Taxa Accounted For _______ (initials) Data Entry (initials)___________ Entry Date _____/______/______

Continue on Back YES / NO (circle one) Page ____ of ____

Figure 3. Example of bench data sheet used to record identified benthic macroinvertebrates. ID, identification; hr(s), hour(s); Prep, preparation; LS = life stage.

Table 1. Dimensions of subsampling equipment used in the quantitative processing of benthic macroinvertebrate samples

[All dimensions in centimeters]

Type	Inside dimensions	Grid dimensions
Subsampling frame		
12 grid	15.2 by 20.3 by 3.8	5.1 by 5.1
24 grid	20.3 by 30.5 by 3.8	5.1 by 5.1
42 grid	30.5 by 35.6 by 3.8	5.1 by 5.1
Estimation tray		
49 grid	8.9 by 8.9 by 1.3	1.3 by 1.3
81 grid	11.4 by 11.4 by 1.3	1.3 by 1.3

2. Sample Preparation

Sample preparation consists of a series of steps that are completed prior to starting the process described herein. Steps include (1) understanding and following safety issues, (2) obtaining supplies, chemicals, and equipment, and (3) washing, sieving, and elutriating samples. All samples are electronically logged in at the NWQL. Sample problems, such as leaking containers and information descrepancies, are resolved with the customer before starting sample processing.

2.1. Safety Issues

2.1.1. Personal safety

An apron, rubber gloves, and protective eyewear are worn during sample preparation. Long pants and closed toe shoes are worn at all times. The nearest eyewash and shower stations are shown to individuals working in the laboratory. They are also instructed in handling chemical and sample spills.

2.1.2. Chemical safety

Exposure to sample preservatives (for example, formalin and ethanol) is minimized by performing the initial washing steps in a fume hood. Organisms are sorted from samples in dishes or trays of water. Liquid and solid wastes are stored in sturdy, chemical resistant containers and discarded by following appropriate local, State, and Federal regulations. Materials Safety Data Sheets for chemicals used or disposed of during sample processing are clearly displayed in the laboratory.

2.2. Obtaining Chemicals, Equipment, and Supplies

Before initiating work on a specific processing task, necessary chemicals, equipment, and supplies are obtained. In doing so, processing efficiency is increased, and the likelihood for analytical error minimized.

2.3. Washing, Sieving, and Elutriating Samples

Within 2 weeks of receiving a sample, the original field preservative (typically 5–10 percent buffered formalin) is decanted through a sieve in a fume hood. The sample is then rinsed with water and preserved with 70-percent ethanol until processed. Preservative exchange is important because some BMIs can become brittle, and the calcareous shells of mollusks can dissolve if they remain in formalin for extended periods, thus making identification to desired levels difficult.

Sieves are used in the laboratory to wash and size-fractionate samples before sorting organisms. Sieve mesh sizes used in processing are based on the mesh size used in sample collection and on specific study objectives. The goal of sample washing is to remove sample preservatives and fine debris (for example, sand and silt), which can obscure the sorting of small BMI organisms. Sieves used for washing have a mesh-size opening less than or equal to the field collection mesh size. BMIs retained on the sieves after processing are removed and

placed with the sample. Sieves are washed and scrubbed before starting another sample.

Some studies (for example, Cuffney and others, 1993b) encourage prior field processing to facilitate sample processing in the laboratory following field collection of a BMI sample (see Appendix 1). Despite extra field processing, however, some samples might require additional laboratory preparation. Samples are often elutriated in the laboratory to remove inorganic sample debris (for example, sand and gravel) before subsampling or sorting. The purpose of this step is to minimize the adverse effects that inorganic debris can have on distributing organic sample debris and organisms evenly in a gridded subsampling frame or sorting tray. Samples are elutriated by carefully swirling the entire sample in a tub of water to suspend the organic debris and organisms. Once suspended, the organic debris is poured slowly into another sieve or wash basin, leaving behind the heavier, inorganic debris. These steps are repeated until the inorganic debris has been separated from the organic matter.

3. Qualitative Visual Sort Method for Processing Benthic Macroinvertebrate Samples

3.1. Application

The goal of the qualitative processing method is to produce a comprehensive list of BMI taxa present in a sample. The abundance of each taxon is not determined.

3.2. Summary of Method

Samples are visually sorted under a light by a taxonomist for up to 2 hours (fig. 4). Samples are first size-fractionated to separate coarse and fine organic debris to increase sorting effectiveness. The coarse-size fraction is sorted for about 0.25 hour, while the fine-size fraction is sorted for up to 1.75 hours. Sorting is focused on mature, undamaged organisms that can produce Genus- or Species-level taxonomic resolution. Immature or damaged specimens are sorted if it is likely that they represent new taxa from the sample. The objective of sorting is to find as many distinct taxa as practical within the 2-hour limit. Studies performed in the BG indicated that the rate of accrual of new taxa diminishes substantially after 2 hours of visual sorting; therefore, the visual sorting period used in the qualitative method is limited to 2 hours (fig. 5).

3.3. Interferences

Sorting effectiveness varies with the type and amount of sample detritus. An excessive amount of organic detritus reduces one's ability to adequately differentiate organisms (especially small, cryptic organisms) from the sample matrix. Large clumps of algal filaments must be carefully separated, and delicate organisms (for example, mayfly larvae) must be carefully handled to minimize damage or loss of taxonomically valuable body parts, such as gills and legs. Consequently, samples with large amounts of organic detritus or filamentous algae are difficult to sort and may have large numbers of damaged specimens.

3.4. Procedure

The ethanol preservative is rinsed from the sample through a sieve that has a mesh size less than or equal to that used in the field. If necessary, the sample is elutriated to separate inorganic and organic detritus. The sample is then size-fractionated by using a 4.75-mm sieve. To ensure consistent and effective sorting, the sample is apportioned evenly among multiple white sorting trays. The number and size of the trays are adjusted so that about 50 percent of the bottom is visible in each tray.

Total sorting time is limited to 2 hours. The coarse-size fraction is sorted for about 0.25 hour. The remaining time, about 1.75 hours, is apportioned between the fine-size fraction and any elutriated inorganic debris (fig. 4); however, if the taxonomist determines that the entire sample has been adequately sorted without adding different taxa, then sorting is terminated at less than 2 hours. This action is approved by a second taxonomist and noted on the bench data sheet. If the volume of the fine-size fraction is such that it cannot be adequately sorted in about 1.75 hours, then the sample is divided

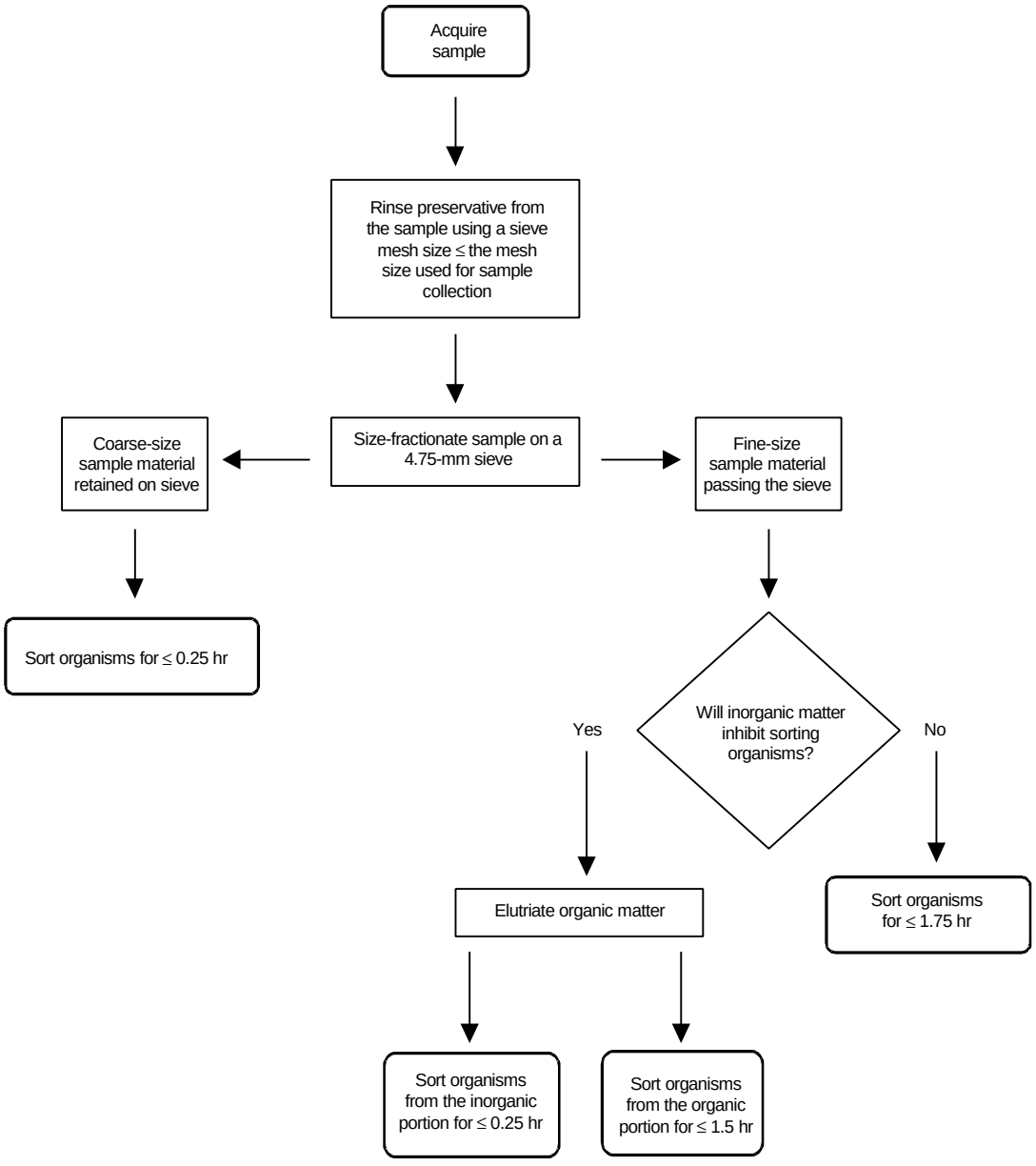


Figure 4. Overview of the qualitative, visual sort method for processing benthic macroinvertebrate samples. mm, millimeter; hr, hour(s); =, less than or equal to.

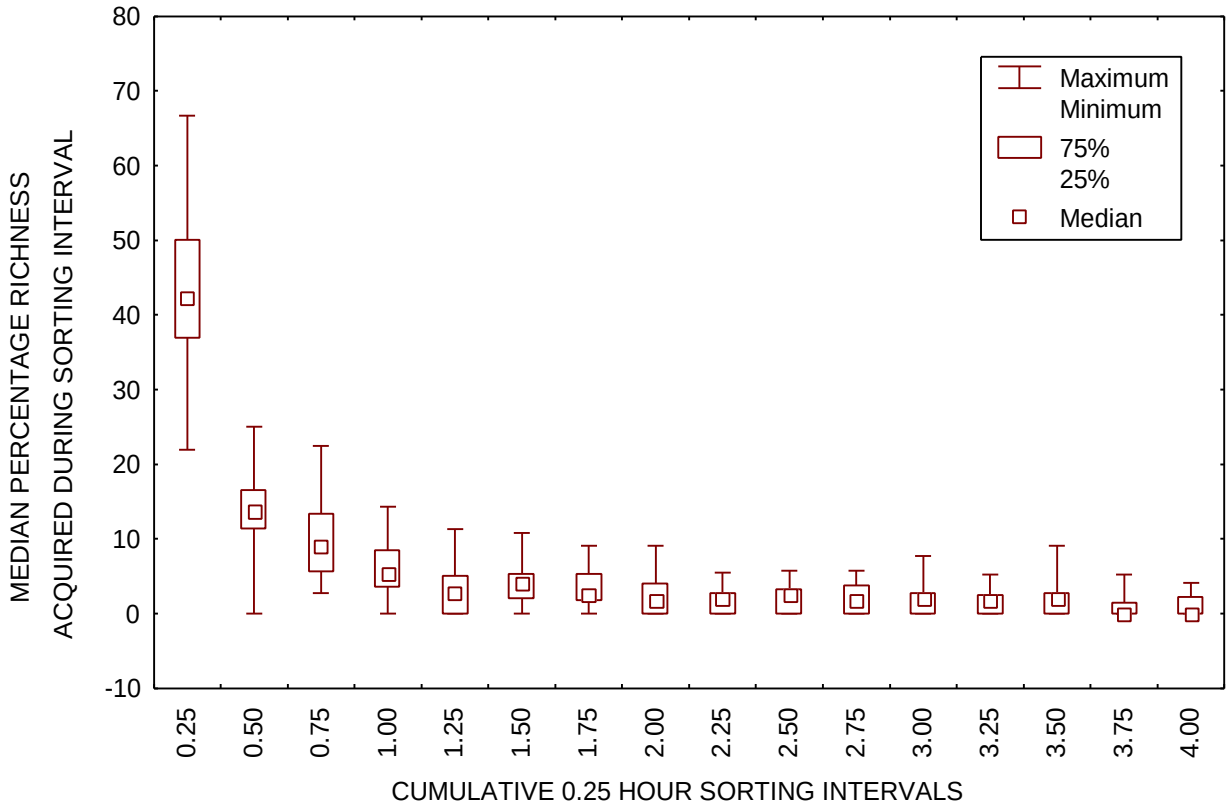


Figure 5. Median percentage benthic macroinvertebrate richness acquired at 0.25-hour intervals over a 4-hour period using the qualitative, visual sort processing method. Percentage (%) richness is based on the maximum 4-hour richness (n=16).

directly on a sieve or on an appropriate subsampling frame so that at least 25 percent of this fine-size fraction can be sorted. The remaining unsorted remnant is quickly scanned and sorted for distinct taxa.

Each tray is sorted systematically by a taxonomist for mature, undamaged organisms. After one complete pass of the tray, the detritus is redistributed by rocking the tray and sorting continues. BMIs are sorted into gross taxonomic categories (table 2) and placed into polyseal screw-cap vials that contain 70-percent ethanol. At least 50 Chironomidae larvae are sorted whenever possible. Visually distinguishing Genus- or Species-level diversity for some BMI taxa (for example, hydropsychid caddisflies and elmid beetles) is often difficult. Therefore, comparable numbers of organisms of these groups are sorted from each tray of each sample. All unique mollusk shells are sorted, even if the body of the organism is not present.

3.5. Qualitative Selection of Chironomidae Larvae for Slide Mounting

All larvae are mounted for samples where less than or equal to 50 larvae are sorted. Where greater than 50 larvae are originally sorted, about 50 larvae are culled to maximize the number of different taxa mounted on slides. Specimens are selected for mounting on the basis of morphological characters diagnostic of common subfamilies (table 3). The objective is to maximize the number of midge taxa identified by selecting and mounting organisms with as many different combinations of diagnostic characters as possible.

3.6. Quality Control of Sorting Effectiveness

After at least 25 percent of the sample has been sorted, a second taxonomist scans the sorted remnant for obviously missed or under-represented taxa for about 0.25 hour to ensure that the sample is sufficiently sorted. This QC step is performed before the completion of sorting so that recommendations can be implemented, while the taxonomist sorts the remainder of the sample.

Table 2. Taxonomic categories used for benthic macroinvertebrate sorting

Taxonomic sorting categories
Gastropoda (snails)
Bivalvia (clams)
Oligochaeta (segmented worms)
Hirudinea (leeches)
Hydrachnidia (water mites)
Decapoda (crayfish/shrimp)
Amphipoda/Isopoda (scuds/sow bugs)
Ephemeroptera (mayflies)
Odonata (dragonflies/damselflies)
Plecoptera (stoneflies)
Heteroptera (true bugs)
Megaloptera (dobsonflies/fishflies/alderflies)
Trichoptera (caddisflies)
Lepidoptera (moths)
Coleoptera (beetles)
Diptera (true flies)
Chironomidae (midges)
Others (nematodes, flatworms)

4. Quantitative Fixed-Count Method for Processing Benthic Macroinvertebrate Samples

4.1. Application

The fixed-count method is normally used to process BMI samples that have been collected using a quantitative or semiquantitative sampling method (for example, sampling standardized by unit area or volume). However, the fixed-count method can also be used to produce estimates of relative abundance of the taxa sorted from qualitatively collected samples (for example, sampling standardized by unit effort).

Table 3. Morphological characters used to select larvae qualitatively for slide preparations from Chironomidae subfamilies

	Morphological character			
Subfamily	**Antennae**	**Ligula**	**Ventromental plates**	**Shape of head capsule**
Chironominae	Nonretractile	Absent	Well developed/ striated	Round
Diamesinae	Nonretractile/ annulated	Absent	Reduced	Round/square
Orthocladiinae	Nonretractile	Absent	Reduced	Round/square
Tanypodinae	Retractile	Present	Absent	Square

4.2. Summary of Method

The principal objective of the fixed-count method is to identify and estimate the abundance of each BMI taxon sorted from the sample. This method is similar to the USEPA's RBP sample-processing procedure (Barbour and others, 1999; Plafkin and others, 1989). The fixed count is based on a minimum number of organisms sorted from the sample and is defined by the study's data-quality objectives (for example, 100-, 200-, or 300-organism fixed-count target).

Samples containing more organisms than the fixed-count target are subsampled by using a subsampling frame partitioned into 5.1- by 5.1-cm grids. However, uniformly distributing a sample in a subsampling frame is often difficult, and organisms in the sample matrix tend to have a clumped distribution (table 4). Therefore, subsampling by simply acquiring a single, very small portion from a subsampling frame could lead to extreme errors in estimating the abundance of taxa in the sample. The method described below uses multiple, randomly selected 5.1- by 5.1-cm portions of the original sample (stage-1 grids) to estimate abundance accurately. Large-rare organisms are sorted from any remaining portion(s) of the sample after the random subsampling is complete.

Total sorting time is limited up to a maximum of 8 hours, depending on the fixed-count target. The time limitation has been implemented to avoid spending too much time on samples that contain few organisms (for example, equal to or less than the fixed-count target) or have exceedingly difficult detritus to sort (for example, filamentous algae).

A generalized processing procedure is shown in figure 6 and listed as follows:

- The sample is uniformly distributed in a subsampling frame (stage-1 subsampling frame).
- An estimate of the average number of organisms per stage-1 grid is obtained.
- By using the average number of organisms per stage-1 grid, an appropriate processing strategy is selected.
- The grids are randomly selected from either a stage-1 or a stage-2 subsampling frame, and organisms are sorted from each grid.
- Large-rare organisms are sorted from any remaining unsorted portion(s) of the sample.

Table 4. Index of dispersion summary statistics used to determine the distribution of benthic macroinvertebrate organisms in samples spread across subsampling frames

[Data are from National Water-Quality Assessmet Program Study Unit samples (CAZB, Central Arizona Basin; LINJ, Long Island-New Jersey; SANT, Santee Cooper Basin; UCOL, Upper Colorado River Basin); ID, identification; No., number; S^2, variance; df, degrees of freedom; Index, index of dispersion statistic; χ^2, Chi-square statistic. Chi-square superscripts denoting type of distribution are defined as "r" (random) or "c" (clumped). Shaded blocks indicate that 2-stage subsampling was not performed.]

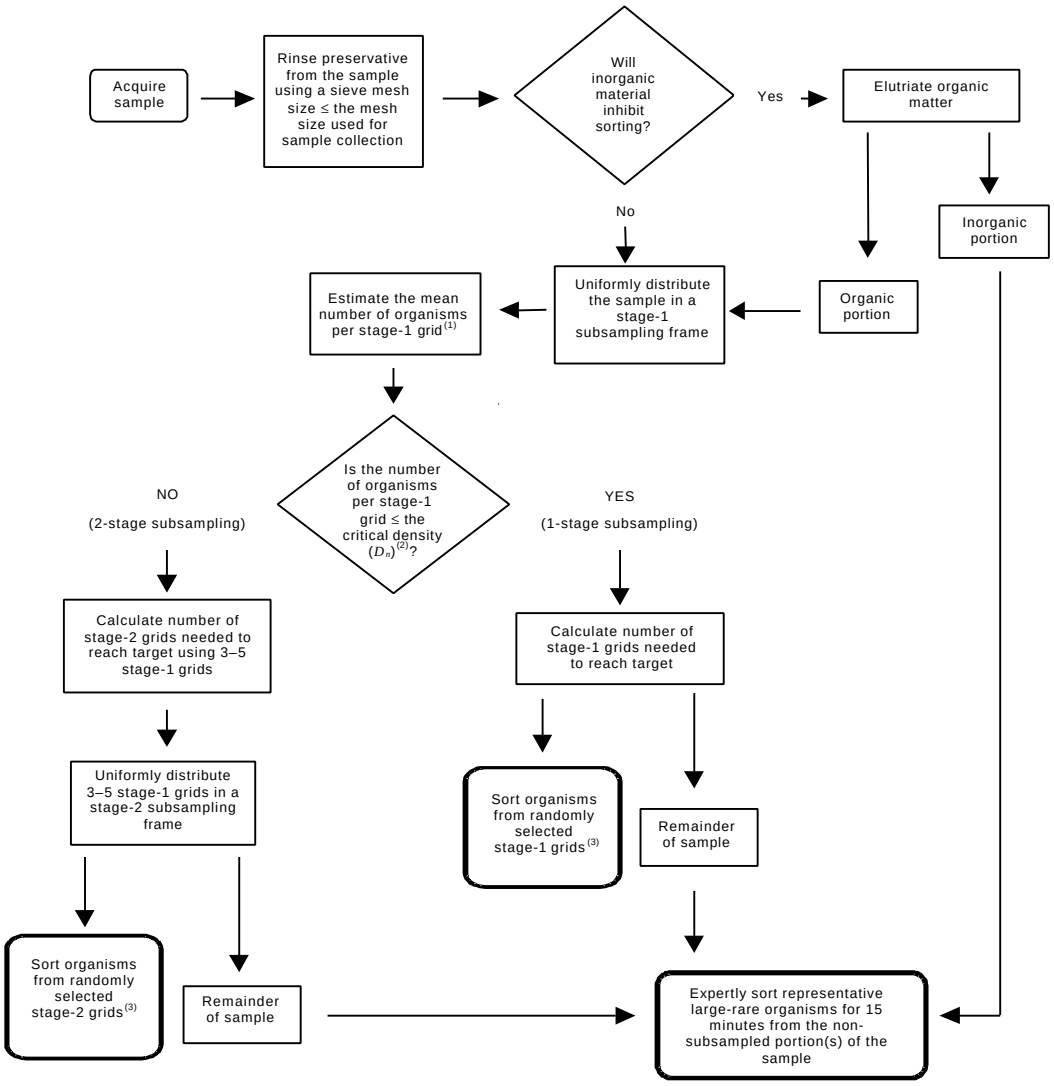
	1-stage subsampling		2-stage subsampling							Confidence limits	
Sample ID	No. grids sorted or transferred for 2-stage subsampling	No. grids in frame	No. grids sorted	No. grids in frame	S^2	Mean No. organisms per grid	df	Index	χ^2	0.025	0.975
CAZB1	7	42	5	12	123.00	75.0	4	1.64	6.56^r	0.484	11.143
CAZB2	5	24			353.20	52.8	4	6.69	26.76^c	0.484	11.143
CAZB3	5	42	5	12	4267.30	173.6	4	24.58	98.32^c	0.484	11.143
CAZB4	5	42	5	12	2677.30	148.6	4	18.02	72.07^c	0.484	11.143
LINJ1	5	42	5	24	244.00	92.0	4	2.65	10.61^r	0.484	11.143
LINJ2	5	42	5	42	312.20	119.8	4	2.61	10.42^r	0.484	11.143
LINJ3	5	42	5	42	1389.70	165.8	4	8.38	33.53^c	0.484	11.143
LINJ4	5	42	5	12	673.70	127.2	4	5.30	21.19^c	0.484	11.143
SANT1	12	42			151.15	33.3	11	4.53	49.88^c	3.816	21.920
SANT2	5	24			485.20	81.2	4	5.98	23.90^c	0.484	11.143
SANT3	5	24			102.70	89.8	4	1.14	4.57^r	0.484	11.143
SANT4	7	42			353.57	58.7	6	6.02	36.13^c	1.237	14.449
UCOL1	5	24	5	12	37.70	74.8	4	0.50	2.02^r	0.484	11.143
UCOL2	5	24	5	42	198.50	84.0	4	2.36	9.45^r	0.484	11.143
UCOL3	5	42			22.70	27.8	4	0.82	3.27^r	0.484	11.143
UCOL4	6	42			268.57	81.2	5	3.31	16.54^c	0.831	12.833
UCOL5	8	42			166.84	52.6	7	3.17	22.19^c	1.690	16.013

4.2.1. Choosing a subsampling frame

Unlike many subsampling devices, gridded frames are useful for subsampling a variety of difficult sample matrices (for example, filamentous algae). Three sizes of subsampling frames are used (see table 1). The size of the subsampling frame chosen depends on the total sample volume and organism density; frame size increases with sample volume and density (table 5). If the volume of a sample is very low but the density of the BMIs is high, the subsampling frame size is dictated by the density of organisms in the sample. Occasionally, the volume of detritus is so small and the BMIs are so depauperate that the use of a subsampling frame is not necessary. The primary objective is to choose a frame size for uniform dispersal of the sample.

4.2.2. Estimating the mean number of organisms per stage-1 grid

The mean number of organisms per stage-1 grid is used to determine the appropriate subsampling strategy. This mean is obtained by randomly selecting five grids from the stage-1 subsampling frame and uniformly distributing the material from each grid into separate, appropriately sized, estimation trays. Estimation trays with either 49 or 81 grids (table 1) can be used to obtain a uniform distribution and density of sample material. The organisms in each of three randomly chosen estimation tray grids are counted and used to estimate the number of organisms in each estimation tray and, hence, each stage-1 grid. Separate estimates are made from each of the five estimation trays. The resulting five estimates



(1) The mean number of organisms per subsampling frame is determined by using estimation trays that subsample each of five stage-1 grids.
(2) See table 6 for D_{300} and D_{100}
(3) At least 3 grids are always sorted. The maximum number of grids sorted is determined by numeric (fixed-count) and time criteria. Grids are sorted in their entirety until the fixed-count or processing-time criteria are exceeded.

Figure 6. Overview of the quantitative, fixed-count method for processing benthic macroinvertebrate samples. (≤, less than or equal to.)

Table 5. Suggested stage-1 subsampling frame sizes used for various sample volumes

[sample volume in milliliters; <, less than]

Sample volume	Subsampling frame size
< 250	12 grids
250 – 500	24 grids
500 – 750	42 grids

are averaged to give an estimate of the number of organisms in each stage-1 grid (see Section 4.4.1).

An informed processing decision can be made once the mean number of organisms per stage-1grid has been estimated. Subsampling may involve processing multiple randomly selected stage-1 grids from the stage-1 subsampling frame (1-stage subsampling) or a further subsampling of three to five stage-1 grids (2-stage subsampling). Numeric criteria are used to determine the appropriate subsampling strategy (see Section 4.4.2). Once the appropriate level of subsampling has been achieved, the approximate number of random 5.1- by 5.1-cm grids are randomly selected for sorting. Additional grids are randomly selected as needed to reach the fixed-count target.

4.2.3. Sorting organisms

The contents of each randomly chosen stage-1 or stage-2 grid are sorted separately by using a dissecting microscope with X 10 magnification. All identifiable organisms are sorted (see Section 1.1). Mollusk shells are only sorted if the animals are present in the shells. Only a portion of colonial organisms, such as Bryozoa or Porifera, is sorted to document its presence in the sample. Vertebrates, exuviae, invertebrate eggs, microcrustaceans, and terrestrial organisms are not sorted. However, terrestrial insects that have an aquatic lifestage (for example, adult mayflies and caddisflies) are sorted.

Once sorting has begun, the grid is sorted to completion even if numeric or time criteria are exceeded. Organisms are enumerated as they are removed from each grid and pre-sorted into the categories listed in table 2. Organisms are placed in polyseal capped vials containing 70-percent ethanol. The sort-time criteria, excluding time required to prepare the sample and estimate grid densities, are 8 hours for a 300-organism fixed-count target and 3 hours for a 100-organism fixed-count target.

4.2.4. Sorting large-rare organisms

Some large-rare taxa (for example, clams, stoneflies, hellgrammites) may be present but at such low densities that it is unlikely that they will be encountered in the random subsamples. These organisms often represent long-lived and ecologically important taxa that should be included in water-quality studies. Therefore, the quantitative sample-processing method accounts for these large-rare taxa by visually sorting them from the unsorted portion of the sample. This sorting is limited to 15 minutes. If inorganic debris was separated from the sample (see fig. 6), this debris also is sorted for large-rare organisms.

4.3. Interferences

Inorganic debris in the sample matrix interferes with the uniform distribution of the sample matrix in the subsampling frame. Substantial amounts of inorganic debris are separated from the sample matrix by elutriation before distributing the organic portion of the sample in the subsampling frame (fig. 6). Large organic detritus is removed, rinsed, inspected for attached organisms, and then discarded. Samples that contain substantial amounts of filamentous algae are distributed as evenly as possible. The algae are cut by using scissors to aid in removing randomly selected grids from matrices that contain filamentous algae.

A large sample matrix also inhibits efficient subsampling and sorting. The total volume of most samples collected from about 1 m^2 can be sufficiently field processed to reduce the submitted volume of the sample to < 750 mL. Laboratory splitting is some-

times necessary if the total submitted sample volume exceeds 750 mL.

4.4. Procedure

4.4.1. Estimating mean organism abundances in the 5.1- by 5.1-cm stage-1 grids using the estimation trays

The mean number of organisms in a 1.3- by 1.3-cm estimation tray grid (B) is first determined by averaging the number of organisms in each of three randomly chosen estimation tray grids (A_i):

$$B = \frac{1}{3}\sum_{i=1}^{3} A_i \ . \qquad (1)$$

The estimated number of organisms in each stage-1 grid (C_i) is subsequently determined from each of five estimation trays, as follows:

$$C_i = e \times B \ , \qquad (2)$$

where e = 49, if the 8.9- by 8.9-cm estimation tray is used, or e = 81, if the 11.4- by 11.4-cm estimation tray is used.

The mean number of organisms per stage-1 grid (D) is then calculated as follows:

$$D = \frac{1}{5}\sum_{i=1}^{5} C_i \qquad (3)$$

The value of D is used to determine an appropriate subsampling strategy.

4.4.2. Determining the specific processing strategy

The fixed-count and time criteria for quantitative sample processing can be achieved in different ways. For example, the criteria can be achieved by processing different numbers of stage-1 grids (1-stage subsampling) and by subsequent subsampling of a subset of the stage-1 grids (2-stage subsampling). The number of combinations that could be used is large, so it would be possible to apply substantially different processing procedures to samples with similar numbers of organisms. A more standard approach for determining when and how a sample should be subsampled is highly desirable. Therefore, sample-processing procedures have been developed on the basis of the average density per grid in the stage-1 subsampling frame (table 6). The sample-processing procedures in table 6 were selected so that no fewer than three randomly selected subsample grids are sorted. No fewer than three grids are sorted because the distribution of organisms within a subsampling frame may be clumped (table 4). The following process also strives to achieve total sorted organism counts only slightly in excess of the target.

The procedure for processing a sample to target a 300-organism fixed count begins by evaluating the average number of organisms per stage-1 grid (D).

Table 6. Processing procedures used to reach to reach 300- or 100-organism fixed-count targets

[cm, centimeter; =, less than or equal to; <, less than; >, greater than]

<table>
<tr><th rowspan="3">Estimated organism density per grid in the stage-1 subsampling frame (D_n)[1]</th><th colspan="6">Subsampling frame sizes (in number of 5.1- by 5.1-cm grids)</th></tr>
<tr><th colspan="3">Number of stage-1 grids to sort</th><th colspan="3">Number of stage-2 grids to sort</th></tr>
<tr><th>12</th><th>24</th><th>42</th><th>12</th><th>24</th><th>42</th></tr>
<tr><td>D_{300} £ 120
D_{100} £ 40</td><td>3–12</td><td>3–24</td><td>3–42</td><td colspan="3"></td></tr>
<tr><td>120 < D_{300} £ 216
40 < D_{100} £ 72</td><td colspan="3" rowspan="2">2-stage-subsampling by using five stage-1 grids</td><td>4–6</td><td colspan="2" rowspan="2"></td></tr>
<tr><td>216 < D_{300} £ 432
72 < D_{100} £ 144</td><td colspan="2">4–7</td></tr>
<tr><td>432 < D_{300} £ 1008
144 < D_{100} £ 336</td><td colspan="3"></td><td colspan="2">3–6</td></tr>
<tr><td>1008 < D_{300} £ 1260
336 < D_{100} £ 420</td><td colspan="3">2-stage subsampling by using four stage-1 grids</td><td colspan="2">3–4</td></tr>
<tr><td>1260 < D_{300} £ 1680
420 < D_{100} £ 560</td><td colspan="3">2-stage subsampling by using three stage-1 grids</td><td colspan="2">3–4</td></tr>
<tr><td>D_{300} > 1680
D_{100} > 560</td><td colspan="6">Additional subsampling is necessary</td></tr>
</table>

[1] D_{300} corresponds to a 300-organism fixed-count target; D_{100} corresponds to a 100-organism fixed-count target

1. If $D \leq 120$, then 2-stage subsampling is not necessary.

The extent of 1-stage subsampling is determined by calculating the estimated total number of stage-1 grids (rounded to the nearest integer ≥ 3) needed to reach the fixed-count target (E). E is determined as follows:

$$E = \frac{300}{D}, \quad (4)$$

If E is greater than or equal to the number of grids in the stage-1 subsampling frame, then the entire sample is sorted (for as much as 8 hours). Otherwise, E randomly selected stage-1 grids are sorted. Processing begins with the five originally chosen stage-1 grids used to determine D. If fewer than five grids are needed, then the first three or four stage-1 grids chosen are sorted. If more than five grids are needed, then additional stage-1 grids are chosen at random from the stage-1 subsampling frame and sorted.

2. If D > 120, then 2-stage subsampling is necessary.

Performing 2-stage subsampling involves randomly selecting three to five stage-1 grids, uniformly redistributing material from these stage-1grids onto a stage-2 subsampling frame, and then randomly selecting a subset of grids (stage-2 grids) to sort from the stage-2 subsampling frame. The number of stage-1 grids that are combined and placed in the stage-2 subsampling frame, the size of the stage-2 subsampling frame,

and the estimated number of stage-2 grids that are combined to obtain the stage-2 subsample are all based on a series of calculations.

The number of organisms in an aggregation of three, four, or five stage-1 grids (G_i) is determined as follows:

$$G_i = i \times D \quad (5)$$

where i = 3, 4, or 5 stage-1 grids; D = the average number of organisms per stage-1 grid.

The estimated number of stage-2 grids (H_k) to be sorted to reach the fixed-count target is then determined for the available stage-2 subsampling frames as follows:

$$H_k = \frac{300}{G_i / k} \quad (6)$$

where k = 12, 24, or 42 (that is, the stage-2 subsampling frame size).

Whenever possible G_5 should be used in the calculation of H_k. Values of H_k are always rounded up to the nearest integer and should be greater than or equal to 3 and less than or equal to 7. However, some stage-1 subsampling frames may have too high of a density (D) to achieve an H_k greater than or equal to 3 and less than or equal to 7, when using G_5. In these cases, G_4 followed by G_3 should be used in the calculation of H_k.

When multiple H_k's are valid for a given G_i, then the estimated number of organisms that would be sorted from H_k stage-2 grids (I_k) may be calculated to aid in choosing k as follows:

$$I_k = H_k \times \frac{G_i}{k} \quad (7)$$

where $3 \le H_k \le 7$.

The value of I_k can be compared to the fixed-count target and used to select the most appropriate combination of i (the number of stage-1 grids combined and placed in the stage-2 subsampling frame) and k (the stage-2 subsampling frame size). Whether or not I_k is used to select the most appropriate subsampling strategy, the i randomly selected stage-1 grids are recombined and uniformly distributed on the appropriately sized stage-2 subsampling frame (k). The standard subsampling decisions made by the BG are listed in table 6.

These calculations consider the original organism density, the size of the stage-2 subsampling frame, the fixed-count target, and the estimated number of organisms in the final (stage-1 or stage-2) subsample. This procedure can produce a fixed-count subsample slightly in excess of 300 organisms from a sample containing < 70,560 organisms. If the estimated number of organisms contained in the sample exceeds 70,560, the sample must be processed differently.

4.4.3. Determination of laboratory correction factor

If a sample is subsampled in the laboratory, a laboratory subsampling correction factor is calculated (table 7). The laboratory correction factor is recorded on (1) the Subsampling and Preliminary Enumeration Worksheet and (2) the Identification and Enumeration Bench Data Sheet as *a:b*, where *a* is the combined numerator and *b* is the combined denominator (table 7).

Table 7. Calculation of the laboratory subsampling correction factor

[W = total grids in the stage-1 subsampling frame; X = total grids sorted from the stage-1 subsampling frame; Y = total grids in the stage-2 subsampling frame; Z = total number of grids sorted from the stage-2 subsampling frame]

	Subsampling strategy	
	1-Stage subsampling	**2-Stage subsampling[1]**
Laboratory subsampling correction factor (*L*)	$L = \frac{W}{X}$	$L = \frac{W}{X} \times \frac{Y}{Z}$

[1]When 2-stage subsampling, X will typically be 5.

4.4.4. Determination of field-correction factor

If the submitted sample was subsampled in the field, the abundance of each taxon is corrected for field subsampling by applying a field-correction factor (F) as calculated below:

$$F = \frac{V_{collected}}{V_{submitted}}\ , \qquad (8)$$

where $V_{collected}$ = total volume of sample collected in the field; $V_{submitted}$ = total volume of sample submitted for processing.

4.5. Quality Control

4.5.1. Sorting effectiveness

The primary purpose of re-sorting is to detect and then correct sorting error, as for example, (1) to discover a subsample grid that was inadvertently missed during the initial sorting effort or (2) to sort taxa that are systematically overlooked. Sorting effectiveness is determined by re-sorting the sorted sample remnant.

To detect sorting errors, the remnant of every sample is re-sorted at X 10 magnification by a second taxonomist for at least 10 percent of the time that the sample was originally sorted. All organisms recovered are added to the original sort vials and become a permanent part of the sample. The total number of organisms obtained during the re-sorting period is recorded on the estimation worksheet, and percentage sorting effectiveness (E_S) is calculated as follows:

$$E_S = 100 \cdot \frac{S}{R+S}, \qquad (9)$$

where R = the total organisms obtained during the re-sort of the grid remnants, and S = the total organisms originally obtained from the sorted grids. It is expected that ≥ 80 percent of the organisms be removed during the original sort.

New taxonomists are evaluated by using a more stringent sorting effectiveness procedure. Sorting effectiveness checks are performed on all grids as they are sorted for at least the first five samples processed by a new taxonomist and no time limit is imposed. The purpose of this procedure is to ensure that sorting standards and operational issues are understood before new taxonomists begin to process samples on their own. After achieving the sorting standards (typically after processing five samples), new taxonomists are evaluated by using the normal sorting effectiveness procedures.

4.5.2. Documentation

After a sample has been sorted, a second taxonomist confirms the recorded accuracy of the subsampling strategy and the resulting correction factors. This task is performed by initialing the appropriate space on the Subsampling and Preliminary Enumeration Worksheet (fig. 1).

5. Slide Preparations

5.1. Application

Some BMI taxa (for example, chironomid larvae and worms) are best identified to the Genus- or Species-level by using a compound microscope after they have been cleared and permanently mounted on microscope slides.

5.2. Summary of Method

Organisms are oriented in mounting media (for example, CMC-10™) on a microscope slide that is labeled with a unique sample identifier and slide number, covered with a cover slip, and dried overnight at 55°C.

5.3. Interferences

Poor slide mounts of organisms often prevent a taxonomist from making identifications to the Genus or Species level. Factors contributing to this problem include (1) improper orientation of the organism on the slide, (2) mounting organisms too numerous or large for one cover slip, or (3) complications with the clearing action and viscosity of the mounting media.

5.4. Procedure

Organisms are sorted into morphologically similar groups by using a dissecting microscope. One drop of mounting media is placed on a slide and spread to approximate the area of a cover slip (maximum two cover slips per slide). Organisms are blotted on a paper towel to remove excess fluids and oriented in the mounting media in the same direction to allow optimal viewing of diagnostic structures. No more than four organisms are mounted under each cover-slip. Mounting medium is added where necessary to compensate for the size and number of organisms being mounted. A cover slip is placed over the organisms by laying one side against the slide and carefully lowering it over the organisms. Application of slight directional pressure to the cover slip is often required to orient organisms or to remove air bubbles in the mounting media. Prepared slides are dried overnight at 55°C. Slides are checked periodically for void spaces; if necessary, additional mounting medium is added to the edge of the cover slip. Dried slides are stored on their sides in boxes grouped by project according to the sample identification code.

5.4.1. Special instructions for mounting Chironomidae

Chironomidae are grouped according to life stage (larvae or pupae), size, and subfamily. Larvae are oriented vertically on the slide with heads to the top. Heads and thoraxes of large larvae (for example, Diamesinae) are mounted separately from the abdomen. Heavily sclerotized larval heads are mounted separately from the rest of the body. Directional pressure may need to be applied to cover slips to rotate larvae so that the ventral sides of heads are visible. Pupae are oriented dorsal side up.

5.4.2. Instructions for mounting worms

Worms are grouped according to Family and size, then mounted on their sides. Individual worms are oriented horizontally on the slide with heads to the left.

6. Taxonomic Identification of Benthic Macroinvertebrates

6.1. Application

Taxonomic identification of BMIs depends upon experienced personnel trained in zoological taxonomic principles and having a broad knowledge of all aquatic macroinvertebrate groups. Typically dichotomous keys are used to identify organisms, which offer a formal, stepwise method for arriving at a name for an organism based primarily on its morphological characteristics. Progression through the dichotomous key results in classification of the organism according to a nomenclatural hierarchy (for example, Order? Family? Genus? Species) of increasing morphological similarity. It is desirable to achieve the lowest level of taxonomic classification possible (for example, Species) because ecological characteristics and responses to water-quality conditions are more specific at lower taxonomic

levels (for example, Species) than at higher levels (for example, Genus or Family) (Resh and Unzicker, 1975). However, identification to Species is not always possible because of maturity, condition of the specimen, or the current state of taxonomic knowledge about a group of organisms.

Identifying BMIs can require viewing the whole organism under low magnification by using a dissecting miscroscope or it can require clearing and mounting an organism (or its parts) on a microscope slide for viewing at high magnification by using a compound microscope (for example, Chironomidae larvae). Different tissue clearing and mounting techniques are required that depend on the size and type of organism. Chironomidae larvae are generally mounted in a viscous medium (for example, CMC-10) that renders body tissues transparent. Other organisms may require dissection and clearing of body parts in a cold or hot solution of potassium hydroxide (for example, insect genitalia) to facilitate viewing. The cleared organism or body parts are mounted temporarily in glycerin and examined under a dissecting or compound microscope. Adult identification keys might require familiarity with wing, reproductive, and other adult morphological characters.

6.2. Interferences

Most larval identification keys, unless otherwise stated by their authors, are constructed on the basis of morphological characters that are found in mature larvae. In practice, many organisms collected in field samples are either too immature (for example, early instar larvae) or are damaged during collection, shipping, and laboratory processing. Consequently, the morphological characters required to identify the organism are often missing or obscured, and the identification of an organism is frequently terminated at a higher taxonomic level than desired (for example, Class, Order, or Family instead of Genus or Species). Consequently, higher level determinations are justified on the bench data sheet to facilitate the interpretation of taxonomic data used for analyses. The BG uses several standardized supporting notes for this purpose (table 8), including others that convey additional information about the determination (table 9). Even though determinations to the recommended levels are not always possible, BMI taxonomists, who are familiar with regional or local faunas, the taxonomic literature, and have access to a verified reference collection, can sometimes make a determination at a lower taxonomic level. The BG also uses several standardized provisional or conditional designations (table 10) to convey as much taxonomic information as possible when the taxonomy of a group is incomplete or unclear, or when a potentially undescribed taxon has been discovered.

6.3. Taxonomic Information Resources

Taxonomy is a dynamic process. Species new to science are continually being described and previous designations revised, thereby requiring the construction of new identification keys and re-examination of the validity of some species. As a result, taxonomic identifications are checked against the most current and widely accepted list of names for a particular group to ensure their validity and use. Concomitantly, BG taxonomists are required to stay current with the taxonomic literature, access reference collections, and interact with recognized specialists.

6.3.1. Taxonomic literature

An extensive taxonomic library is used at the NWQL to support the identification of BMI taxa. The BMI taxonomic literature is diverse and widely scattered among many peer-reviewed journals, books, and newsletters. Major types of taxonomic literature include the following: descriptions, reviews and revisions of taxa (for example, Moulton and others, 1999), taxonomic monographs of regional faunas (for example, Baumann and others, 1977; Brigham and others, 1982; Moulton and Stewart, 1996), checklists (for example, Moulton and Stewart, 1997), and major continental treatments (for example, Merritt and Cummins, 1996;

Table 8. Standardized notes used to justify benthic macroinvertebrate identifications where the prescribed taxonomic level is not achieved

Note	Description
imm.	➢ Means “immature” and includes all synonyms thereof ➢ Identificaton to prescribed level not supported because the organism(s) is/are too immature ➢ May be applied to larvae or pupae
dam.	➢ Means “damaged” and includes all synonyms thereof ➢ Identification to prescribed level not supported because the organism(s) is/are damaged
mount	➢ Means “poor mount” and includes all synonyms thereof ➢ Identification to targeted level not supported because slide mounted organism(s) is/are poorly oriented on slide
indet.	➢ Means “indeterminate” and includes all synonyms thereof ➢ Identification to targeted level not supported for recently molted organisms, mayfly subimagos, mature and intact organisms because of undocumented variation or indistinct characters, required case is missing/damaged, or required habitat/ecological information is missing/unavailable ➢ Unlikely that taxon is new to science
gender	➢ Includes males and females ➢ Identification to targeted level not supported because of gender
retained	➢ Denotes unmounted/unidentified organisms retained in separate vial

Table 9. Notes of taxonomic interest that convey additional information about benthic macroinvertebrate identifications

Note	Description
new state record	➢ Refers to a potential new state record for a taxon based on known distributional information in the published literature or other reliable source
new U.S. record	➢ Refers to a potential new United States record for a taxon based on known distributional information in the published literature or other reliable source
new species ?	➢ Represents a potentially undescribed species that cannot be linked to any closely related species ➢ Used with Genus-level identification only
no. lost	➢ Refers to the number of organisms accidentally lost in handling ➢ The number of organisms lost is indicated before “lost” ➢ Example: 2 lost, ? lost, all lost
artifact	➢ Identification of a bryozoan fragment (missing zooids) or empty mollusk shell ➢ Only used in qualitatively processed samples, when taxon is not represented by a complete organism
ref.	➢ Denotes an organism(s) placed in a reference collection

Table 10. Standardized conditional or provisional taxonomic designations applied to benthic macroinvertebrate identifications

Designation	Description
sp.	➢ Species place holder for identifications to Genus-level only ➢ Denotes both singular and plural forms of species ➢ Example: *Hydropsyche* sp.
sp. nr.	➢ Means "species near" ➢ Refers to a potentially undescribed species nearest to the species/authority following the designation ➢ Example: *Hydropsyche* sp. nr. *simulans* Ross
cf.	➢ Means "confer" ➢ Refers to a species that closely matches the species/authority following the designation but differs morphologically in some minor ways or the description in the literature is too vague or incomplete to be certain ➢ Example: *Hydropsyche* cf. *simulans* Ross
/ "slash"	➢ Used to denote two or more taxa that are unresolvable or where only two species are known in a monophyletic group ➢ Placed between the taxa in question ➢ Taxa are ordered alphabetically ➢ If Species, authorities are included ➢ Example: *Hydropsyche rossi* Flint, Voshell, and Parker/*simulans* Ross
sp. 1 or sp. A genus A	➢ Refers to provisional taxa reported in the literature where their specific identity remains unknown; also known as "operational taxonomic units" or "OTUs" ➢ Provisional designation is reported exactly as it appears in the literature ➢ Provisional designation is followed parenthetically by the author(s) and year of the publication ➢ Example: *Oecetis* sp. A (Floyd, 1995)
group	➢ Denotes a group of more than two closely related species that cannot be separated or organisms that can be reliably placed in a species group where determination to species is unsupported ➢ If only two species in the group, then use "/" or slash designation ➢ Is formally recognized in the literature ➢ Example: *Hydropsyche scalaris* group
complex	➢ Denotes a species for which there may be considerable variation suggesting two or more cryptic species ➢ Is formally recognized in the literature ➢ Example: *Oecetis inconspicua* complex
n. sp.	➢ Means "new species" ➢ Represents a species new to science that has been verified by a recognized authority or one that appears in the literature as such ➢ If the designation appears in the literature, the designation must be followed parenthetically by the authors and year of the publication ➢ Example: *Hydroptila* n. sp. (Moulton and Stewart, 1997)
Other conditional or provisional designations	➢ Reported exactly as they appear in the reference from which they were obtained ➢ The designation is followed parenthetically by the author(s) and year of the publication ➢ Example: *Stilocladius*? sp. (Epler, 1995)

Stewart and Stark, 1988; Wiggins, 1996). In addition, BG taxonomists consult taxonomic and distributional information that is available on the Internet. A list of useful taxonomic references and articles for identification of BMIs is presented in Appendix 2.

6.3.2. Reference collection

A reference collection of BMIs is maintained at the USGS NWQL that associates one or more actual specimens with each taxonomic name. This collection helps to ensure that future taxonomic comparisons are accurate and consistent. This collection is North American in scope and includes representative taxa identified from BMI samples collected throughout the United States. Referenced taxa are noted on the bench data sheet (see table 9). Preference for selecting reference taxa is given first to organisms that are mature, intact, and, when possible, are available in a series (several organisms of a taxon in a single sample) from a particular sample. Organisms may be selected despite their condition if they represent the only verifiable record of a particular taxon. Taxa are also referenced from as many different geographic regions as possible to provide distributional information or to assess potential morphological variation across their range. Referenced taxa are verified by a second BG taxonomist or recognized specialist before they are added to the reference collection.

6.3.3. Taxonomic specialists

Taxonomic specialists outside the BG are consulted to assist with problematic taxonomic issues or to confirm identifications. Specialists are experts in their area of taxonomic interest and have a demonstrated record of peer-reviewed publication in taxonomy, systematics, and biogeography of BMIs. The BG consults with the most appropriate taxonomic specialist to verify the identification of threatened and endangered (T&E) BMI species. If a T&E species is confirmed, the BG contacts the appropriate State and Federal authorities regarding the presence and disposition of the T&E species.

6.4. Taxonomic Procedures

6.4.1. Levels of taxonomic assessment

The BG provides three levels of taxonomic assessment for BMI samples. These levels include (1) the Standard Taxonomic Assessment (STA), (2) the Rapid Taxonomic Assessment (RTA), and (3) the Custom Taxonomic Assessment (CTA). Each provides a different basic level of taxonomic resolution to address various water-quality and related data-analysis objectives. The STA and RTA are adapted from the U.S. Environmental Protection Agency (USEPA) Rapid Bioassessment Protocols (RBP) (Barbour and others, 1999; Plafkin and others, 1989).

The STA (table 11) represents a taxonomic effort similar to that described in the USEPA RBP III (Barbour and others, 1999; Plafkin and others, 1989) and in many other state biomonitoring protocols. It is currently (2000) the level of resolution used by the USGS NAWQA Program for BMI samples. In general, mollusks, crustaceans and insects are identified to either the Genus or Species level. Aquatic worms are identified to the Family level. Other BMI groups, such as flatworms and nematodes, are typically identified at higher taxonomic levels (for example, Phylum or Class). By providing lower level taxonomic identification for most BMI groups, the STA allows investigators to consider more detailed analyses that rely on Species-specific ecological and environmental affinities between BMIs and the physical and chemical attributes of their habitats.

The RTA represents a taxonomic effort similar to the USEPA RBP II (Barbour and others, 1999; Plafkin and others, 1989) (table 12). In general, all BMI groups are identified to the Family level, except for groups such as flatworms and nematodes, which are typically identified at higher taxonomic levels (for example, Phylum or Class). The RTA represents a higher level of

Table 11. Levels of benthic macroinvertebrate taxonomic identification specified in the Standard Taxonomic Assessment

Taxon	Level of identification	Taxon	Level of identification
Porifera	Family	Corduliidae	Genus/Species
Cnidaria	Family/Genus	Gomphidae	Genus/Species
Platyhelminthes	Class	Libellulidae	Genus/Species
Nematoda	Phylum	Macromiidae	Genus/Species
Nemertea	Genus	Petaluridae	Genus/Species
Nematomorpha	Phylum		
Bryozoa	Phylum	**Plecoptera**	
Gastropoda	Genus	Capniidae	Genus
Bivalvia	Genus	Chloroperlidae	Genus
Polychaeta	Family	Leuctridae	Genus
Aphanoneura	Family	Nemouridae	Genus
Oligochaeta	Family	Peltoperlidae	Genus
Hirudinea	Family	Perlidae	Genus/Species
Hydrachnidia	Order	Perlodidae	Genus/Species
Amphipoda	Genus	Pteronarcyidae	Genus/Species
Isopoda	Genus	Taeniopterygidae	Genus
Decapoda	Genus		
Collembola	Order	**Heteroptera**	
		Belostomatidae	Genus/Species
Ephemeroptera		Corixidae	Genus
Acanthametropodidae	Genus/Species	Gelastocoridae	Genus
Ameletidae	Genus	Gerridae	Genus/Species
Ametropodidae	Genus/Species	Hebridae	Genus
Arthropleidae	Genus/Species	Hydrometridae	Genus
Baetidae	Genus/Species	Macroveliidae	Genus/Species
Baetiscidae	Genus/Species	Mesoveliidae	Genus
Behningiidae	Genus/Species	Naucoridae	Genus
Caenidae	Genus/Species	Nepidae	Genus/Species
Ephemeridae	Genus/Species	Notonectidae	Genus
Ephemerellidae	Genus/Species	Ochteridae	Genus
Heptageniidae	Genus/Species	Pleidae	Genus
Isonychiidae	Genus	Saldidae	Genus
Leptohyphidae	Genus/Species	Veliidae	Genus
Leptophlebiidae	Genus/Species		
Metretopodidae	Genus/Species	**Megaloptera**	
Neoephemeridae	Genus/Species	Corydalidae	Genus/Species
Oligoneuriidae	Genus/Species	Sialidae	Genus
Polymitarcyidae	Genus/Species		
Potamanthidae	Genus/Species	**Neuroptera**	
Pseudironidae	Genus/Species	Sisyridae	Genus
Siphlonuridae	Genus/Species		
		Trichoptera	
Odonata		Apataniidae	Genus/Species
Calopterygidae	Genus/Species	Beraeidae	Genus
Coenagrionidae	Genus/Species	Brachycentridae	Genus/Species
Lestidae	Genus/Species	Calamoceratidae	Genus/Species
Protoneuridae	Genus/Species	Dipseudopsidae	Genus
Aeshnidae	Genus/Species	Ecnomidae	Genus/Species
Cordulegastridae	Genus	Glossosomatidae	Genus/Species

Table 11. Levels of benthic macroinvertebrate taxonomic identification specified in the Standard Taxonomic Assessment—Continued

Taxon	Level of identification	Taxon	Level of identification
Trichoptera—Continued		Hydrophilidae	Genus
Goeridae	Genus/Species	Hydroscaphidae	Species
Helicopsychidae	Genus/Species	Lampyridae	Family
Hydrobiosidae	Genus/Species	Limnichidae	Genus
Hydropsychidae	Genus/Species	Lutrochidae	Genus/Species
Hydroptilidae	Genus/Species	Melyridae	Family
Lepidostomatidae	Genus	Microsporidae	Genus
Leptoceridae	Genus/Species	Noteridae	Genus
Leptoceridae	Genus/Species	Ptilidae	Family
Limnephilidae	Genus/Species	Psephenidae	Genus
Molannidae	Genus/Species	Ptilodactylidae	Species
Odontoceridae	Genus	Salpingidae	Family
Philopotamidae	Genus/Species	Scirtidae	Family
Phryganeidae	Genus/Species	Staphylinidae	Family
Polycentropodidae	Genus/Species	Tenebrionidae	Family
Psychomyiidae	Genus/Species		
Rhyacophilidae	Genus/Species	**Diptera**	
Rossianidae	Genus/Species	Athericidae	Genus
Sericostomatidae	Genus/Species	Blephariceridae	Genus
Uenoidae	Genus/Species	Canacidae	Genus
Xiphocentronidae	Genus/Species	Ceratopogonidae	Genus
		Chaoboridae	Genus
Lepidoptera		Chironomidae	Subfamily/Tribe/Genus
Arctiidae	Genus	Corethrellidae	Genus
Cosmopterigidae	Genus	Culicidae	Genus
Nepticulidae	Genus	Deuterophlebiidae	Genus
Noctuidae	Genus	Dixidae	Genus
Pyralidae	Genus	Dolichopodidae	Family
Tortricidae	Genus	Dryomyzidae	Genus
		Empididae	Genus
Coleoptera		Ephydridae	Family
Amphizoidae	Genus	Muscidae	Family
Anthicidae	Family	Nymphomyiidae	Genus
Carabidae	Family	Pelecorhynchidae	Genus
Chrysomelidae	Family	Phoridae	Family
Curculionidae	Family	Psychodidae	Genus
Dryopidae	Genus/Species	Ptychopteridae	Genus
Dytiscidae	Subfamily/Tribe/Genus	Sarcophagidae	Family
Elmidae	Genus/Species	Scathophagidae	Family
Epimetopidae	Genus	Sciomyzidae	Genus
Georyssidae	Genus	Simuliidae	Genus
Gyrinidae	Genus/Species	Stratiomyidae	Genus
Haliplidae	Genus	Syrphidae	Family
Helophoridae	Genus	Tabanidae	Genus
Heteroceridae	Family	Tanyderidae	Family
Histeridae	Family	Thaumaleidae	Family
Hydraenidae	Genus	Tipulidae	Family/Genus
Hydrochidae	Genus		

Table 12. Levels of benthic macroinvertebrate taxonomic identification specified in the Rapid Taxonomic Assessment

Taxon	Level of identification
Porifera	Family
Cnidaria	Family
Platyhelminthes	Class
Nematoda	Phylum
Nemertea	Genus
Nematomorpha	Phylum
Bryozoa	Phylum
Gastropoda	Family
Bivalvia	Family
Polychaeta	Family
Aphanoneura	Family
Oligochaeta	Family
Hirudinea	Family
Hydrachnidia	Order
Amphipoda	Family
Isopoda	Family
Decapoda	Family
Insecta (except Collembola)	Family
Collembola	Order

taxonomic effort (for example, Family) compared to the STA; it can be used to screen large numbers of sampling sites for the detection of initial or gross water-quality impairment.

The CTA provides a customer-specified taxonomic effort that is not provided in the STA or RTA. For example, even though oligochaete worms are identified to family in the STA, they can be identified to Genus or Species in the CTA. Customers interested in the CTA should contact the BG to discuss their taxonomic requirements because Species-level resolution for some BMI groups is either extremely difficult or impossible.

6.4.2. Reporting of results

Following identification of BMIs, each taxon is listed on the bench data sheet along with its life stage [if applicable, for example, L=larva(e), P=pupa(e), A=adult(s)] and supporting taxonomic note(s) where applicable. Species-level identifications are reported for monotypic genera. Each identified taxon is placed in a 4–6 dram vial(s) containing 70-percent ethanol along with a taxonomic identification label. Vials of identified BMIs are inventoried against the taxonomic names listed on the bench data sheet to check for unrecorded names and to ensure that each name listed is represented by at least one organism.

In general, all complete and fragmented BMIs are enumerated if at least the head is present. Fragmented or incomplete heads are not enumerated. Although mollusks are frequently identified to Genus or Species by using shell characteristics, at least the organism must be present for the taxon to be identified and enumerated in quantitatively processed samples. Molluscan shells without the organism are identified in qualitatively processed samples and noted accordingly (table 9) on the bench data sheet.

A majority of the morphological characters used to identify pupal and adult insects are located on the terminal abdominal segments (for example, genitalia). In most cases, these segments must be present to achieve low-level taxonomic resolution. For this reason, insect pupae and adults are identified and enumerated provided that at least the terminal abdominal segments and some portion of the thorax are present. In order to avoid a potentially redundant record, head and thorax combinations from pupal and adult insects are only enumerated if at least some of the anterior abdominal segments are present as well. No attempt is made to match fragments with the remainder of the body. Organism parts that are dissected or inadvertently fragmented during identification are stored in a ¼-dram shell vial or microvial containing 70-percent ethanol, plugged with cotton and placed in the taxon vial. Larval sclerites from pupal metamorphotypes are either placed in the puparium or in a microvial.

6.5. Quality Control

6.5.1. Verification of taxonomic identifications

The BG uses an approach to verifying taxonomic identifications that simultaneously checks the accuracy of identifications and the precision of individual taxonomists. The

approach consists of verifying a random selection of 10 percent of all BMI taxa identified by laboratory taxonomists on a weekly basis. In addition, all taxa representing new or unverified additions to the BG master taxonomic list are included in this review. A QC Officer verifies all taxa, and in doing so, might consult with other taxonomists. This approach is followed because it provides a more comprehensive evaluation of the performance among taxonomists for taxonomic identifications in all samples where the selected taxa are found. Since taxonomic errors are either isolated (single occurrences) or systemic (multiple regular occurrences), this approach allows for more appropriate decisions to be made regarding the diagnosis and correction of taxonomic errors. As a result, consistency in taxonomic identification is maintained among BG taxonomists and samples.

6.5.2. Review of benthic macroinvertebrate data

The taxa chosen for taxonomic verification are also re-enumerated in quantitatively processed samples to determine the accuracy of the original count. As general guidance, differences in enumeration for each BMI taxon are maintained within the enumeration limits specified in table 13. Enumeration differences that result from changes in the level of identification following taxonomic verification are not assessed as enumeration errors.

Table 13. Performance limits used to evaluate the enumeration of benthic macroinvertebrates

[+, plus; ±, plus or minus; %, percent]

Actual count for a given taxon in the sample		Acceptable deviation from the recorded value
Lower limit	Upper limit	
1	5	±0
6	15	±1 organism
16	35	±2 organisms
36	55	±3 organisms
56	85	±4 organisms
86+		±5% rounded up

6.5.3. Corrective actions

Bench data sheets are reviewed for completeness before data entry. For all instances where required information is missing (for example, the count for a taxon or a life stage), the taxa are re-evaluated as needed and the data sheets are corrected. In addition, all identifications without a supporting note (see table 8) that have not been identified to the prescribed level of assessment are re-evaluated.

6.5.4. Verification of benthic macroinvertebrate enumerations

The QC Officer examines all errors involving identification, enumeration, and bench-data-sheet completeness and determines what corrective actions are necessary. Errors and necessary corrections are reviewed with the taxonomist prior to addressing them. The QC Officer performs a follow-up review to determine that all corrections are made.

7. Data Management

BMI data are entered into a computer spreadsheet and reviewed for accuracy and completeness. Taxonomic names are checked for spelling errors and compared against the BG BMI hierarchy to determine their validity. Data are also reviewed to ensure that supporting information, such as life stage, taxonomic notes, enumerations, and correction factors, is recorded where necessary.

BMI taxa are arranged in phylogenetic order with unprocessed abundances corrected for any laboratory and field subsampling performed (= sample abundance). Unprocessed abundances and organism densities (number of organisms/m^2) are provided on request. Data are typically released in a tab-delimited ASCII format usable by common spreadsheet and data-base software packages (table 14). Data for individual samples are distinguished by the sample identification code (sample ID). A current copy of the BG BMI hierarchy is made availablle with each data set released to facilitate analysis of the taxonomic data.

Table 14. Example benthic macroinvertebrate data set for a quantitative sample

[Taxa are arranged phylogenetically; ID, identification code; BG, Biological Group; LS, life stage; ref., reference collection; sp., species; L, larva(e); A, adult(s); dam., damaged; imm. immature; indet., indeterminate]

Sample ID	BG determination	LS	Notes	Sample abundance
Sample #1	Turbellaria			50
Sample #1	Nematoda			75
Sample #1	*Leptoxis carinata* (Bruguière)		ref.	128
Sample #1	*Physa* sp.			25
Sample #1	*Corbicula* sp.			25
Sample #1	Hydrachnidia			176
Sample #1	Cambaridae		gender	25
Sample #1	Caenidae	L	dam.	25
Sample #1	*Drunella* sp.	L		25
Sample #1	*Serratella deficiens* (Morgan)	L		76
Sample #1	*Tricorythodes* sp.	L		25
Sample #1	Baetidae	L	imm.;dam.	605
Sample #1	*Acentrella turbida* (McDunnough)	L		76
Sample #1	Heptageniidae	L	imm.;dam.	605
Sample #1	*Leucrocuta* sp.	L		151
Sample #1	*Stenonema mediopunctatum* (McDunnough)	L		25
Sample #1	*Isonychia* sp.	L		378
Sample #1	*Stylogomphus albistylus* (Hagen)	L		25
Sample #1	*Acroneuria* sp.	L		2
Sample #1	*Neoperla* sp.	L		1
Sample #1	*Agnetina* sp.	L		2
Sample #1	*Corydalus cornutus* (Linnaeus)	L		5
Sample #1	*Sialis* sp.	L		25
Sample #1	*Chimarra* sp.	L		328
Sample #1	Hydropsychidae	L	imm.	630
Sample #1	*Ceratopsyche* sp.	L		2,092
Sample #1	*Ceratopsyche* cf. m*orosa* (Hagen)	L		252
Sample #1	*Cheumatopsyche* sp.	L		25
Sample #1	*Hydropsyche leonardi* Ross	L	new state record	101
Sample #1	*Microcylloepus* sp.	L		50
Sample #1	*Microcylloepus pusillus* (LeConte)	A		25
Sample #1	*Optioservus* sp.	L		50
Sample #1	*Optioservus trivittatus* (Brown)	A		76
Sample #1	*Stenelmis crenata* group	A		328
Sample #1	Stenelmis sp.	L		1,411
Sample #1	*Psephenus herricki* (DeKay)	L		27
Sample #1	*Bezzia/Palpomyia* sp.	L		25
Sample #1	*Microtendipes* sp.	L		151
Sample #1	*Polypedilum* sp.	L		302
Sample #1	*Rheotanytarsus* sp.	L		403
Sample #1	*Stempellinella* sp.	L		76
Sample #1	Orthocladiinae	L	indet.	25
Sample #1	*Cricotopus* sp.	L		25
Sample #1	*Tvetenia* sp.	L		504
Sample #1	*Thienemannimyia* group sp.	L		101
Sample #1	Simuliidae	L	imm.	378
Sample #1	*Tipula* sp.	L		1
Sample #1	*Atherix lantha* Webb	L		25
Sample #1	*Tabanus* sp.	L		1

SUMMARY

The Biological Group (BG) of the National Water Quality Laboratory processes benthic macroinvertebrate (BMI) samples by using consistent and well-defined methods. The BG has the capability to perform taxonomic identifications on aquatic invertebrate fauna collected from throughout the United States. BMI taxonomic and abundance data can be used in aquatic ecological and water-quality assessments.

The BG qualitatively processes BMI samples by using a visual sort method. The objective of this method is to produce a comprehensive and accurate list of unique taxa sorted from a sample. This method includes sorting a size-fractionated sample component and systematically sorting all or some part of the remainder of the sample. Total sorting time is limited to 2 hours.

The BG quantitatively processes BMI samples with a method that uses numeric (fixed-count) and time (total sorting time) criteria in a method similar to the U.S. Environmental Protection Agency Rapid Bioassessment Protocols. Organisms are either sorted from the entire sample or, more often, from randomly selected subsamples of the original sample. The BG method differs from the Rapid Bioassessment Protocol method by (1) targeting a minimum number of organisms, (2) performing a large-rare organism sort on the unsorted part of the sample, (3) limiting sorting effort to a maximum of 8 hours, and (4) sorting samples under a dissecting scope at X 10 magnificaton.

The National Water Quality Laboratory BG provides three levels of taxonomic assessment: (1) Standard Taxonomic Assessment, (2) Rapid Taxonomic Assessment, and (3) Custom Taxonomic Assessment. The Standard Taxonomic Assessment represents a Genus/Species approach for most taxa. The Rapid Taxonomic Assessment reduces taxonomic effort by identifying BMIs to the Family level and higher. Other taxonomic levels not provided in the Standard or Rapid Taxonomic Assessments are provided when possible with the Custom Taxonomic Assessment, depending on the customer's data-quality objectives.

Sample processing and taxonomic identification is quality assured by using consistent and well-defined quality-control procedures. All sorted sample remnants are resorted to determine sorting effectiveness. A random 10 percent of the identificatons completed weekly (across projects and taxonomists) are reviewed for accuracy. In addition, all names new to the master taxonomic list are verified internally before being placed in a reference collection. Taxonomic specialists' external to the BG may be consulted to verify taxa or to assist in resolving complex taxonomic issues.

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APPENDIXES

Appendix 1.—Benthic macroinvertebrate sample qualifiers

BMI samples can be processed onsite to create several different sample components. The extent of this process depends not only on decisions made onsite at the time of sample collection but on the subsequent laboratory processing methods desired. The NAWQA program produces up to four different sample components for each BMI sample collected (Cuffney and others, 1993b). A brief description of each of these components is presented here.

Main-body component

The main-body sample component represents the majority of the organic detritus collected with the sample and does not exceed 750 mL in volume (excluding preservative). Large material, such as rocks, twigs, macrophytes, and large aggregations of filamentous algae, are gently washed in the field, inspected for attached macroinvertebrates, and then discarded.

Large-rare component

A large-rare component is produced for a sample when large and rare specimens (for example, crayfish, mussels, or hellgrammites) are present in the original sample. This component should contain only a few carefully selected specimens. These specimens are removed before any field subsampling and placed in a sample container separate from the remaining detritus. They are segregated to minimize damage to them or to other, more delicate specimens during initial field preservation, field subsampling, or shipment to the laboratory.

Elutriate component

An elutriate component is produced if inorganic debris (for example, sand or pebbles) are present in the sample. Elutriation involves swirling the sample in a bucket followed by careful decanting of the suspended organic detritus and organisms into a sieve or bucket. The heavier inorganic debris remains in the bucket. Inorganic debris is inspected for case-building caddisfly larvae and mollusks, then discarded onsite or sent to the laboratory for a separate qualitative evaluation.

Split component

A split component is produced onsite when the total volume of the original sample exceeds 750 mL. Sample-splitting procedures are described in Cuffney and others (1993b). This component is assumed to be similar to the main-body component. Split components are retained for later processing if the integrity of the main-body component is compromised during shipping or laboratory processing.

Appendix 2.—List of taxonomic references by major taxonomic groupings

The following list of taxonomic references organized by major BMI groups does not attempt to represent an exhaustive resource for the identification of BMIs. The list contains references deemed important and useful in the taxonomic work performed by the BG. Although checklists, original taxonomic descriptions (or primary literature), and some unpublished works represent important sources of information to a taxonomist, they are not listed here. Also, there is a great deal of taxonomic information available on the Internet. Some of this information has been previously peer reviewed and published and then posted on the Internet for easier access. Other information (for example, checklists) may not have been peer reviewed and is updated with varying frequency. Users should verify the reliability of the sources and read any accompanying qualifying statements or disclaimers.

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